TRADE SECRET

Study Title

H-27529: Local Lymph Node Assay (LLNA) in Mice

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.2600 (2003)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 429) (2001)

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STUDY COMPLETED ON: June 9, 2006

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-19897

WORK REQUEST NUMBER: 16573

SERVICE CODE NUMBER: 1234

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the items documented below. None of the items listed impact the validity of the study.

1. As requested by the sponsor, the study was conducted using test substance that was not characterized.

2. The vehicle control, positive control, and positive control vehicle were not characterized. However, they are commercially available products.

3. The test substance and control preparations used in the study were not analyzed for concentration, uniformity, or stability. The procedures used by trained staff to prepare the dosing preparations ensured:

   - the accuracy of concentration because all preparations were performed using calibrated pipettes,
   - uniformity and stability because each preparation was formulated daily just prior to dosing, and
   - each vehicle and positive control group gave expected results in the study.

Applicant / Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Denise Hoban 09 June 2006
Denise Hoban, B.A, MLT (ASCP) Date
Staff Medical Technologist and Supervisor

Applicant / Sponsor: _____ _____
DuPont Representative Date

QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 16573
Study Code Number: 1234

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	April 14, 2006	April 17, 2006	April 24, 2006
Conduct:	April 21, 2006	April 21, 2006	April 21, 2006
Report/Records:	May 30, 2006	May 30, 2006	June 6, 2006

Reported by: Annet L. Reigel 8 JUN 2006
Annet L. Reigel Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by: Carol Finlay 8 June 2006
Carol Finlay, B.A. Date
Senior Staff Toxicologist

Issued by Study Director: Denise Hoban 09 June 2006
Denise Hoban, B.A, MLT (ASCP) Date
Staff Medical Technologist and Supervisor

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF APPENDICES

Page

STUDY INFORMATION

Substance Tested:	• Crude Industrial Grade HFPODA • H-27529
Haskell Number:	27529
Composition:	85.4-85.8 wt% Balance is water
Purity:	See composition, above
Physical Characteristics:	Clear liquid
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Study Initiated/Completed:	April 13, 2006 / (see report cover page)
Experimental Start/Termination:	April 19, 2006 / April 25, 2006

SUMMARY

The objective of this study was to evaluate the potential of H-27529 to produce a dermal sensitization response in mice using the local lymph node assay (LLNA). Five groups of 5 female CBA/JHsd mice were dosed for 3 consecutive days with 0% (vehicle control), 10%, 25%, 50%, or 100% H-27529 on both ears. Propylene glycol was used as the diluting vehicle. One group of 5 female mice was dosed for 3 consecutive days with 25% hexylcinnamaldehyde (HCA) in 4:1 acetone:olive oil (AOO) as a positive control and one group of 5 female mice was dosed for 3 consecutive days with AOO as a positive control vehicle. On test day 5 of the assay, mice received ^{3}H-Thymidine by tail vein injection and were sacrificed approximately 5 hours later. The cell proliferation in the draining auricular lymph nodes of the ears from the test substance groups was then evaluated and compared to the vehicle control group.

No statistically significant differences in mean body weights compared to the vehicle control group were observed at any test concentration. Statistically significant decreases in mean body weight gains compared to the vehicle control group were observed at the 100% test concentration and positive control vehicle groups.

Following 2 applications of the test substance, one mouse from the 100% test concentration exhibited signs of lethargy, ruffled fur, dehydration, wet fur (ventral). This mouse was later found dead. Bright, red lungs were observed during gross pathology.

Following 3 applications of the test substance, 2 mice from the 50% test concentration were found dead. Gross pathology findings were bright, red lungs and no abnormality detected, respectively.

During the course of the study, 2 mice from the 50% test concentration and 2 mice from the 100% exhibited signs of wet fur, perineum. Additionally, one of these also had bilateral hair loss of the forelimb.

Statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at the 25%, 50%, and 100% test concentrations. Stimulation indexes of greater than 3.0 were observed at the 50% and 100% test concentrations of H-27529. The EC3 value (the estimated concentration required to induce a threshold positive response, i.e., stimulation index = 3) for the test substance under the conditions of this study was calculated to be 37%. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with H-27529. Under the conditions of this study, H-27529 produced a dermal sensitization response in mice.

Based on these data, H-27529 is considered a dermal sensitizer.

INTRODUCTION

The purpose of this study was to examine the dermal sensitization potential of H-27529 using the mouse local lymph node assay (LLNA).[1,2,3,4,5] Following the topical application of the test substance to the dorsal side of both ears, the dermal sensitization potential of the test substance was evaluated by measuring the proliferation of lymphocytes (via radiolabel uptake) obtained from the auricular lymph nodes (i.e., the lymph nodes that drain the ears). Results were compared to the vehicle control group.

Because H-27529 is a liquid and did not appear to have severe skin-irritating capability (pH ~10), the 100% concentration was chosen as the high dose. For subsequent concentrations, the test substance was prepared in propylene glycol (PG).

STUDY DESIGN

The study design was as follows:

Group	Number/ Group	Dosage (%)[a]
II	5	0 (Vehicle Control)
IV	5	10
VI	5	25
VIII	5	50
X	5	100
XII	5	25 (Positive Control)
XIV	5	0 (Positive Control Vehicle)

a % = percent of test substance in vehicle control (e.g., 100% = 1 g/mL, or neat test substance)

Study Parameter	Frequency
Body Weight	Test days 0 and 5
Daily Animal Health Observations	At least once daily
Careful Clinical Observations	Prior to dosing and prior to sacrifice
Dosing	Test days 0-2
Days of Rest	Test days 3-4
Injection of Radioactivity	Test day 5
Removal of Lymph Nodes	At sacrifice (test day 5)
Disintegrations per minute (dpm) data	Test day 6

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.2600: Skin Sensitization, *Health Effects Test Guidelines* (2003)
- OECD, Section 4 (Part 429): Skin Sensitisation: Local Lymph Node Assay, *Guideline for the Testing of Chemicals* (2001)

B. Vehicle Control

The vehicle control, PG, was purchased commercially and used for all test substance dilutions on all dose days. Impurities in the vehicle control were not expected to interfere with the study results. The vehicle control was assumed to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test Substance

The test substance, H-27529, was supplied by the sponsor as a clear liquid. The sample was stored according to the sponsor's instructions. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

The test substance was prepared as a solution in the vehicle control according to the concentrations listed in the Study Design, except for the 100% concentration, which was used neat.

D. Positive Control

The positive control, hexylcinnamaldehyde (HCA), was purchased commercially. Any available information on the positive control was included in the study records. Impurities in the positive control were not expected to interfere with the study results. The positive control appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

A 25% HCA solution in the positive control vehicle was blended using a vortex mixer and stored in a vial protected from light until dosing was completed.

E. Positive Control Vehicle

The positive control vehicle was a 4:1 mixture of acetone:olive oil (AOO). The acetone and olive oil were purchased commercially. Impurities in the positive control vehicle were not expected to interfere with the study results. The positive control vehicle appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

The positive control vehicle was prepared in a clear, glass vial and blended using a vortex mixer.

F. Dosing Preparations and Analyses

Prior to study start, a quantity of the test substance was evaluated for solubility in a particular vehicle. The control and test substance concentrations and method of preparation were based on solubility information. All dose preparations were formulated fresh daily.

Dose preparations were not analyzed for homogeneity or accuracy of concentration. The dose preparation procedures were believed to provide homogeneous mixtures at the targeted concentrations. In the absence of visible change in color or physical state, all dose preparations were assumed to be stable throughout the study.

All dose preparations applied to the test site were assumed to be available for absorption by the test system unless otherwise indicated in the study records. All calculations and the evaluation of effects were based on the applied dose.

G. Test System

On April 11, 2006, 78 female (nulliparous and non-pregnant) CBA/JHsd mice, with an assigned birth date of February 24, 2006, were received from Harlan Sprague Dawley, Frederick, Maryland, U.S.A. Thirty-seven of these mice were randomly selected for this study. The remaining animals were used for other studies or were sacrificed. All mice were approximately 7 weeks old on the day of arrival.

The CBA/JHsd mouse was selected to conduct the LLNA because it is the strain recommended in the test guidelines. In addition, Haskell Laboratory has extensive LLNA experience with the CBA/JHsd mouse strain, and this strain has undergone extensive interlaboratory validation with the LLNA.[1,2,3,4,5]

H. Animal Husbandry

1. Housing

All animals were housed in stainless steel, wire-mesh cages suspended above cage boards. During quarantine, animals were housed in pairs. After assignment to groups, and during the dosing and resting phases of the study, animals were housed singly. After final weighing (test day 5) until sacrifice, animals were housed one group per plastic shoebox cage with appropriate bedding.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26ºC and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

All mice were provided tap water *ad libitum*. All mice were fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*.

4. Animal Health and Environmental Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

I. Pretest Period

Upon arrival at Haskell Laboratory, all mice were:

- quarantined for a minimum of 6 days.
- identified temporarily by the presence or absence of a colored tail mark and cage identification.
- weighed 2 times during quarantine and once prior to initiation of dosing.
- observed with respect to weight gain and any gross signs of disease or injury.

The mice were released from quarantine by the laboratory animal veterinarian or designee on the basis of body weights and clinical signs of all mice.

J. Assignment to Groups

Mice, selected based upon adequate body weight gain and freedom from any ear abnormalities (e.g., torn, scratched) or clinical signs of disease or injury, were distributed into study groups as designated in the Study Design. Prior to study start, each mouse was assigned to a group using a

randomly generated, computer-based algorithm such that individual pretest body weights did not vary more than 20% of the group mean.

At grouping, each mouse was assigned an animal number. The animal number was marked on the tail of each mouse with solvent-resistant ink. Color-coded labels were attached to the animal rack above each cage prior to dosing and included the group number, the animal number, the dose concentration, and the dose substance.

At study start (test day 0), mice were approximately 8 weeks old and weighed between 18.3 and 23.5 grams. On test day 0, when possible, mice with body weights that were not within ± 20% of the mean were removed from study and replaced with mice having body weights within that range (subject to the same selection criteria as the original mice).

Mice not assigned to a test group were released for other laboratory purposes or sacrificed by carbon dioxide asphyxiation and discarded without anatomic pathology evaluation, at the discretion of the study director.

K. Body Weight Measurements

All mice were weighed on test day 0 and prior to sacrifice on test day 5.

L. Clinical Observations and Pathology

Daily animal health observations to detect moribund or dead mice and abnormal behavior and appearance among mice were conducted at least once daily throughout the study. Careful clinical observations were performed prior to each dose and prior to sacrifice by individually handling and examining each animal for abnormal behavior and appearance.

Mice found dead (803 and 805 in the 50% test concentration group and 1001 in the 100% test concentration group) underwent a gross pathology examination.

M. Local Lymph Node Assay

Twenty-five µL of H-27529 were administered topically to the dorsum of each mouse ear for 3 consecutive days (test days 0-2) at dosages listed in the Study Design. One group of mice was similarly dosed with the positive control and one group of mice was similarly dosed with the positive control vehicle. Test days 3-4 were days of rest followed by intravenous injection of 20 µCi of ^{3}H-Thymidine per mouse on test day 5.

Approximately 5 hours after the injection, the surviving animals were sacrificed by carbon dioxide asphyxiation, draining auricular lymph nodes were removed, and single cell suspensions were prepared. The single cell suspensions were incubated at 2-8°C overnight. On test day 6, the single cell suspensions were counted on a beta counter. The counts per minute (cpm) data were converted to disintegrations per minute (dpm).

A stimulation index (SI) was derived for each experimental group by dividing the mean dpm of each experimental group by the mean dpm of the vehicle control group. The decision process in

regard to a positive response includes an SI of greater than or equal to 3.0 together with consideration of dose response and, where appropriate, statistical significance.

N. Statistical Analyses

Significance was judged at $p < 0.05$ except for dpm data that were judged at $p < 0.01$. Lymph node dpm data were transformed to Log to obtain normality or homogenous variances.

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight[a] Body Weight Gain[a]	Levene's test for homogeneity[6] and Shapiro-Wilk test[7] for normality[b]	One-way analysis of variance[8] followed by Dunnett's test[9,10,11]	Kruskal-Wallis test[12] followed by Dunn's test[13]
Lymph Node dpm Data[c]	Test for lack of trend[14]	Sequential application[15] of the Jonckheere-Terpstra trend test[16]	Preliminary tests for pairwise comparison
	OR[d]		
	Levene's test for homogeneity[6] and Shapiro-Wilk test[7] for normality[b]	One-way analysis of variance[8] followed by Dunnett's test[9,10,11]	Kruskal-Wallis test[12] followed by Dunn's test[13]

a Positive control and positive control vehicle data were not included in the statistical analysis of the test substance groups, and were evaluated separately by one-way analysis of variance followed by Dunnett's test.
b If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.
c Positive control and positive control vehicle data were not included in the statistical analysis of the test substance groups.
d Pairwise comparisons and associated preliminary tests were only conducted if the test for lack of trend was significant.

When possible, an EC3 value for the stimulation index data was derived from linear interpolation of points on the dose-response curve immediately above and below the 3-fold threshold. The equation used for calculation of EC3 was:

$$EC3 = c + [(3-d)/(b-d)] \times (a-c)$$

where:

a = the lowest concentration giving stimulation greater than 3
b = the actual stimulation index caused by a
c = the highest concentration failing to produce a stimulation index of 3
d = the actual stimulation index caused by c

RESULTS AND DISCUSSION

A. Body Weights, Body Weight Gains, and Clinical Signs of Toxicity

(Tables 1-3, Appendices A-B)

No statistically significant differences in mean body weights compared to the vehicle control group were observed at any test concentration. Statistically significant decreases in mean body weight gains compared to the vehicle control group were observed at the 100% test concentration and positive control vehicle groups.

Following 2 applications of the test substance, one mouse from the 100% test concentration (1001) exhibited signs of lethargy, ruffled fur, dehydration, wet fur (ventral). This mouse was later found dead. Bright, red lungs were observed during gross pathology.

Following 3 applications of the test substance, 2 mice from the 50% test concentration (803 and 805) were found dead. Gross pathology findings were bright, red lungs and no abnormality detected, respectively.

During the course of the study, 2 mice from the 50% test concentration (801 and 802) and 2 mice from the 100% (1002 and 1003) exhibited signs of wet fur, perineum. Additionally, mouse 1002 had bilateral hair loss of the forelimb.

B. Stimulation Index Data

(Table 4, Appendix C)

Statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at the 25%, 50%, and 100% test concentrations. Stimulation indexes of greater than 3.0 were observed at the 50% and 100% test concentrations of H-27529. The EC3 value (the estimated concentration required to induce a threshold positive response, i.e., stimulation index = 3) for the test substance under the conditions of this study was calculated to be 37%. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with H-27529. Under the conditions of this study, H-27529 produced a dermal sensitization response in mice.

CONCLUSIONS

Based on these data, H-27529 is considered a dermal sensitizer.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

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TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

Mean Body Weights
Mean Body Weight Gains
Summary of Clinical Observations
Stimulation Index Data

dpm - disintegrations per minute
n - number of animals evaluated
N/A - not applicable
S.D. - standard deviation
SI - stimulation index

Table 1
Mean Body Weights of Female Mice

DAYS ON TEST	MEAN BODY WEIGHTS (g)						
	Group II 0%[a]	Group IV 10%	Group VI 25%	Group VIII 50%	Group X 100%	Group XII 25%[b]	Group XIV 0%[c]
0	20.9 1.2(5)	21.0 1.7(5)	21.1 1.3(5)	20.8 1.3(5)	20.8 1.6(5)	21.1 1.4(5)	20.9 1.4(5)
5	21.6 0.7(5)	22.4 2.3(5)	21.9 1.4(5)	19.9 1.2(3)	19.1 1.7(4)	22.4 1.2(5)	21.5 1.4(5)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

a Vehicle control
b Positive control; data were not included in the statistical analysis of the test substance groups, but were evaluated separately.
c Positive control vehicle; data were not included in the statistical analysis of the test substance groups, but were evaluated separately.

There were no statistically significant differences from vehicle control at $p < 0.05$.

Table 2
Mean Body Weight Gains of Female Mice

DAYS ON TEST	MEAN BODY WEIGHT GAINS (g)						
	Group II 0%[a]	Group IV 10%	Group VI 25%	Group VIII 50%	Group X 100%	Group XII 25%[b]	Group XIV 0%[c]
0 - 5	0.7 0.5(5)	1.3 0.9(5)	0.8 0.8(5)	-0.7 0.3(3)	-2.4* 1.1(4)	1.3 0.5(5)	0.6* 0.4(5)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

a Vehicle control
b Positive control; data were not included in the statistical analysis of the test substance groups, but were evaluated separately.
c Positive control vehicle; data were not included in the statistical analysis of the test substance groups, but were evaluated separately.

* Statistically significant difference from vehicle control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.

Table 3
Summary of Clinical Observations

	Group II	Group IV	Group VI	Group VIII	Group X	Group XII	Group XIV
	0%[a]	10%	25%	50%	100%	25%[b]	0%[c]
ANIMAL COUNT:	5	5	5	5	5	5	5
Wet Fur	0 (0%)	0 (0%)	0 (0%)	2 (40%)	3 (60%)	0 (0%)	0 (0%)
Ruffled Fur	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)
Hair Loss	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)
Lethargic	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)
Dehydrated	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)

a Vehicle control
b Positive control
c Positive control vehicle

Table 4
Stimulation Index Data

GROUP	MATERIAL TESTED	n	MEAN (dpm)	S.D. (dpm)	SI
II	0% Vehicle Control	5	261.00	107.85	N/A
IV	10%	5	558.00	338.74	2.14
VI	25%	5	683.20	226.34	2.62#
VIII	50%	3[b]	883.33	258.02	3.38#
X	100%	4[b]	793.00	379.37	3.04#
XII	25% Positive Control[a]	5	2561.00	699.76	6.89
XIV	0% Positive Control Vehicle[a]	5	371.60	135.59	N/A

a Data were not included in the statistical analysis of the test substance groups.
b One or more mice were found dead prior to this analysis and the data for these mice were not evaluated.

Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Jonckheere-Terpstra trend test.

APPENDICES

Appendix A
Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

g - grams

FOOTNOTES:

a This mouse was found dead prior to this analysis and the data for this mouse were not evaluated.

Individual Body Weights

	Body Weight g Day 0	Body Weight g Day 5
Female, II - 0% Vehicle Control		
201	19.4	20.6
202	21.2	21.8
203	20.0	21.3
204	22.3	22.5
205	21.6	21.8
Female, IV - 10% H-27529		
401	18.8	20.4
402	21.1	22.4
403	20.3	20.8
404	23.5	26.2
405	21.4	22.0
Female, VI - 25% H-27529		
601	19.8	21.6
602	21.3	22.2
603	19.9	20.7
604	23.1	24.2
605	21.4	21.0
Female, VIII - 50% H-27529		
801	19.1	18.5
802	20.9	20.4
803	19.9	[a]
804	21.8	20.7
805	22.1	[a]
Female, X - 100% H-27529		
1001	18.3	[a]
1002	21.2	17.3
1003	20.3	18.4
1004	22.7	21.3
1005	21.5	19.2
Female, XII - 25% Positive Control		
1201	19.5	20.9
1202	20.5	22.0
1203	20.4	22.4
1204	23.1	24.1
1205	21.9	22.6
Female, XIV - 0% Positive Control Vehicle		
1401	19.1	19.5
1402	20.7	21.1
1403	20.3	21.5
1404	22.6	23.3
1405	21.9	22.0

Appendix B
Individual Clinical Observations and Mortality Records

Individual Clinical Observations and Mortality Records

Sex	Group	Animal	Observation	Days
F	II	201	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	II	202	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	II	203	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	II	204	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	II	205	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	IV	401	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	IV	402	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	IV	403	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	IV	404	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	IV	405	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VI	601	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VI	602	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VI	603	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VI	604	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VI	605	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VIII	801	General observation, No Abnormality Detected	0-2
			Wet Fur, Perineum	5
			Sacrificed by design	5
F	VIII	802	General observation, No Abnormality Detected	0-2
			Wet Fur, Perineum	5
			Sacrificed by design	5
F	VIII	803	General observation, No Abnormality Detected	0-2
			Found dead in cage.	3
F	VIII	804	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	VIII	805	General observation, No Abnormality Detected	0-2
			Found dead in cage.	3
F	X	1001	General observation, No Abnormality Detected	0
			Lethargic	1-2
			Ruffled Fur	2
			Dehydrated	2
			Wet Fur, Ventral body, Ventral	2
			Found dead in cage.	2
F	X	1002	General observation, No Abnormality Detected	0-1
			Hair Loss, Forelimb, Bilateral	2-5
			Wet Fur, Perineum	5
			Sacrificed by design	5
F	X	1003	General observation, No Abnormality Detected	0-1
			Wet Fur, Perineum	2-5
			Sacrificed by design	5
F	X	1004	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	X	1005	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5

Individual Clinical Observations and Mortality Records

Sex	Group	Animal	Observation	Days
F	XII	1201	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XII	1202	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XII	1203	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XII	1204	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XII	1205	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XIV	1401	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XIV	1402	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XIV	1403	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XIV	1404	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5
F	XIV	1405	General observation, No Abnormality Detected	0-5
			Sacrificed by design	5

Appendix C
Individual Animal Cell Proliferation Data

INDIVIDUAL ANIMAL CELL PROLIFERATION DATA

EXPLANATORY NOTES

ABBREVIATIONS:

dpm - disintegrations per minute

FOOTNOTES:

a This mouse was found dead prior to this analysis and the data for this mouse were not evaluated.

Individual Animal Cell Proliferation Data

Animal	dpm
Female, II - 0% Vehicle Control	
201	162.00
202	317.00
203	423.00
204	213.00
205	190.00
Female, IV - 10% H-27529	
401	396.00
402	384.00
403	323.00
404	1147.00
405	540.00
Female, VI - 25% H-27529	
601	412.00
602	828.00
603	593.00
604	990.00
605	593.00
Female, VIII - 50% H-27529	
801	780.00
802	1177.00
803	[a]
804	693.00
805	[a]
Female, X - 100% H-27529	
1001	[a]
1002	862.00
1003	483.00
1004	1302.00
1005	525.00
Female, XII - 25% Positive Control	
1201	1972.00
1202	3104.00
1203	3284.00
1204	1691.00
1205	2754.00
Female, XIV - 0% Positive Control Vehicle	
1401	486.00
1402	204.00
1403	487.00
1404	248.00
1405	433.00